function, *S. aureus* uses fully saturated but terminally branched lipid acyl chains, which have a significantly lower phase transition temperature than their linear counterparts. We found that lipid vesicles composed of synthetic branched phospholipids are much more susceptible to attack by an amphipathic peptide than lipid vesicles composed of unsaturated phospholipids with the same headgroup composition. By contrast, natural membrane lipid extracts from *S. aureus* produce lipid vesicles that are as resistant to membrane-active peptides as those produced using synthetic, unsaturated lipids. We postulated that an unidentified component present in the *S. aureus* lipid extracts serves to stabilize the bacterial cytoplasmic membrane. A possible candidate is menaquinone, which participates in the bacterial electron transport chain but could, in addition, have a structural effect on the lipid bilayer. In the work presented here, we investigated the kinetics of dye efflux from lipid vesicles containing between 1 and 5 mol% menaquinone, induced by the antimicrobial peptide PMAP-23. PMAP-23 is a 23 amino acid, linear peptide of the cathelicidin family with the sequence RIIDLLWRVRRPQKPKFVTVWVR. We found that the presence of physiological concentrations of menaquinone had a notable effect on peptide-induce dye efflux.

3393-Pos Board B498
In Vivo ^{2}H-NMR Study of the Action of Antibacterial Agents on Escherichia Coli Membranes
Catherine Tardy-Laporte, Alexandre A. Arnold, Isabelle Marcotte.
Contrary to eukaryote membranes, bacterial membranes are negatively charged owing to the presence of phosphatidylglycerol (PG) or cardiolipin in their inner membranes. One of the action mechanisms of antibiotics such as antimicrobial peptides is to selectively interact with negatively charged lipids to create a breach in the bacterial membrane. The characterization of the cellular membrane integrity is, thus, a key element in understanding the mechanism of action of antimicrobial agents. Solid-state nuclear magnetic resonance spectroscopy (SS-NMR) is a useful tool that allows probing the organization and dynamics of phospholipids in a bilayer. In this work, we have performed an *in vivo* ^{2}H-NMRstudy of *Escherichia coli* membranes labeled with deuterated palmitic acid. More specifically, we have studied the effect of nanoparticles (NPs) and antibacterial molecules on the bacteria membrane. The ^{2}H-NMR spectra obtained on intact cells show that the *E. coli* membranes were successfully labeled according to previous work. A 10-hour exposure to the cationic detergent cetyltrimetylammonium chloride (CTAC) shows increased membrane fluidity at high detergent concentration (320μM). The study of the effect of fullerenol reveals that these NPs increase the lipid mobility in the membrane especially after 8 hours of exposure. The ^{2}H-NMR spectra obtained in the presence of polymyxin B show no fast-tumbling membrane fragments, although UV analysis indicates the leakage of the cell content. These results suggest that this antibiotic would perforate *E. coli* membranes without their complete disruption. Our work illustrates the use of *in vivo* ^{2}H NMR studies to understand the specific action of different substances on labeled biological membranes.

3394-Pos Board B499
Regulation of Antimicrobial Peptide Activity through Lipid Chain Order
Diego A. Ramirez, Daniel E. Otzen, Chad Leidy.
During changes in temperature, bacterial membranes present broad but cooperative lipid chain-melting events, where the membrane transitions from a solid-ordered state into a liquid-disordered state. For example, in Staphylococcus aureus, this melting event occurs at 15C. This transition has important implications. When temperature decreases near to the lipid melting event, cell division is inhibited. Gram-positive bacteria present cold-shock response mechanisms that shift the melting event to lower temperatures by varying the saturated fatty acids/unsaturated fatty acids ratio (SFA/UFA). It thus appears that the bacteria adaptatively strives to minimize the solid-ordered phase. However, the solid-ordered phase is not completely detrimental to cell viability. Recently we have shown that the solid-ordered phase induces resistance to PLA2-IIA, an innate human antibacterial agent that acts by disrupting membrane integrity. Therefore, we propose that adaptative modulation of the SFA/UFA ratio of bacterial membranes may alter the physical activity of antimicrobial agents that disrupt membrane integrity. To corroborate this, we studied, with the use of model systems, the activity of two antimicrobial peptides Magainin-2 and Novocidin (AMPs). Based on fluorescence spectroscopy, we measured calcein leakage potency on POPG (unsaturated) / DPPG (saturated) large unilamellar vesicles (LUVs). As we modified the SFA/UFA ratio we observe as expected a shift in the phase transition from ordered to disordered phase. This leads to 1) changes in the peptide needed to induce 50% leakage, and 2) changes in the leakage kinetic rate constant. Furthermore, we relate this kinetic rate to the energetics of a two state model in order to quantify the changes in the activation energy required for AMPs to perturb the bilayer as a function of the SFA/UFA ratio. We relate this change in activation energy to levels of lipid packing by measuring Laurdan generalized polarization.

3395-Pos Board B500
Lipid Bilayers of Ester-Modified Lipids
Diana Y. Villanueva, Joseph B. Lim, Jeffery B. Klauda.
Lipid membranes and bilayers essentially function as barriers for cells to control the transport of substances. Since phospholipid membranes consist of a hydrophilic surface and a hydrophobic inside, the center of a phospholipid bilayer is known to contain almost no water and to prevent the transport of water-dissolving substances, such as ions. These water soluble molecules take alternate routes via ion channel protein pumps to transverse the cell membrane. Typical lipid bilayers with saturated or moderately unsaturated chains show a bilayer with a water density that decreases to zero around 10Å and remains at zero at the center. Recent experimental work suggests that ester-modified lipids allow for ions and water to be present at the center of the bilayer (JACS, 128: 14034). These ester-modified phospholipid bilayers contain ester groups along their hydrocarbon chain at various positions. The addition of the ester groups to these lipids can occur naturally through a free radical reaction called lipid peroxidation. We are using molecular dynamics simulations to study two lipid bilayers with additional ester groups on the chain. One lipid contains ester groups in the upper half of the acyl chain (lipid E) and another contains ester groups towards the middle and the end (lipid G). The bilayers contain 15 lipid G (or E) and 35 POPC lipids per leaflet with a hydration of 70.5 waters per lipid. For lipid E, the water density reaches zero at ~7Å, as oppose to the standard 10Å. As for lipid G, water fully penetrates the bilayer. We are currently extending these simulations to at least 100 ns, to obtain good statistics on ion permeation where longer timescales are needed to further understand this water and ion permeation mechanism.

3396-Pos Board B501
Lipid-Soluble Hydroquinone Modifications Induced on Membranes
Sergio S. Funari, Vivian Rebbin, Liliana Marzorati, Claudio Di Vitta.
We synthesized new alkylthiohydroquinones (ATHs); in order to investigate different aspects of lipid-soluble hydroquinones interactions with phospholipids normally found in cell membranes. They have the same long hydrophobic alky chains found in many lipids forming the cell membranes. In these compounds the tails should share the inner of the membrane, while the hydroquinone, as polar head group should remain on the surface. One or more alkylthio chains attached to the aromatic ring, modifies its hydrophobicity and should alter the electron distribution. Moreover, the two OH groups become chemically distinguishable, e.g. NMR spectroscopy and also show different pKa values.
We investigate the interaction of ATHs with lipid membranes, POPE and POPC and observe the formation of structures with different morphologies, or curvature, of the lipid membrane, depending on temperature and pH. We attributed their formation to changes in the balance charge/polarity induced by the ATHs. Mixtures with POPE at pH=4 forms two cubic phases P4332 and Im3m that reach a maximum lattice size while in basic conditions they only expand upon heating from room temperature. They coexist with lamellar or hexagonal and have been associated with inhomogeneous distribution of the ATHs molecules over the lipid matrix. The zwitterionic POPC does not form cubic phases, but instead shows two lamellar structures up to a high temperature.
In all mixtures of lipid/ATHs we observed the formation of lamellar and hexagonal phases, similar to the behaviour of pure hydrated lipid e.g. POPC, while for POPE additional cubic structures, depending on the environment conditions.

3397-Pos Board B502
N-3 polyunsaturated Fatty Acids Disrupt Micron and Nanometer Scale Non-Raft Organization by Increasing Cell Size and Minimizing Molecular Interactions with Surrounding Rafts
Benjamin Drew Rockett, Andrew Franklin, Mitchel Harris, Heather Teague, Justin Williams, Stephen R. Wassall, Andrew H. Nguyen, Benjamin L. Stottrup, Saame Raza Shaikh.
N-3 polyunsaturated fatty acids (PUFAs), due to their unique molecular structure, modify plasma membrane organization; however, very few mechanistic details are known. Here we tested the hypothesis that n-3 PUFAs, in comparison to other fatty acids, can specifically disrupt the organization of non-rafts on several length scales using quantitative imaging. On a micron scale, EL4 cells treated with eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid robustly increased accumulation of the non-raft probe FAST DiI. The increase in FAST DiI accumulation was dependent on the total cellular levels of EPA and DHA, as revealed by linear regression analysis across differing cell types